Integrating genotypic HIV tropism testing into the Vela Sentosa HIV genotyping and resistance assay

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BACKGROUND

Testing for CCR5-tropic virus is a requirement before starting a Maraviroc containing treatment as this CCR5 blocker can only act against CCR5-tropic HIV. Genotypic tropism testing by analysing the V3 loop sequence is the most common method used nowadays as phenotypic tropism testing is time-consuming and expensive. Genotypic tropism testing on the other side is inexpensive and although a laborious process was needed to define cutoffs and advise for clinical application, today good guidelines and tools exist for interpretation of sequences. To detect and quantify minor non-CCR5-tropic populations the method of choice is to perform tropism testing using next generation sequencing techniques. With some preprocessing this data can be analysed using geno2pheno[454]. We established a method to integrate V3 loop analysis into the Vela Sentosa HIV next generation sequencing genotyping and resistance assay to analyse tropism together with Protease, Reverse Transkriptase and Integrase resistance.

METHODS

For optimized analyses in the geno2pheno coreceptor tool for NGS data (geno2pheno [454]) full length V3-loop sequences are necessary. We designed a nested PCR (1. round V3_6952f GCACAGTACAATGTACACATGG; V3_7357r CAGTAGAAAAATTCCCCTCCAC; 2. Round V3_7062f AATGCCAAAACCATAATAGTACA, V3_7316r TTCTGGGTCCCCTCCTGAG) with a final short PCR product of around 250 basepairs to avoid too much fragmentation within the V3-loop sequence. The PCR-product was spiked into left blank sample cavities after the PCR in the workflow of the Vela Sentosa HIV genotyping and resistance assay. The following steps (library prep, emulsion PCR, enrichment and sequencing) were performed within the standardized Vela workflow. 6636 full length envelope sequences were downloaded from Los Alamos database and used for mapping the NGS data and identifying sequences spanning the complete V3 loop. Using the geno2pheno [454] pre-processor sequences were prepared for upload and analysis with the geno2pheno [454] tool. (Figure 3: MIB NGS tropism pipeline for Vela)

RESULTS

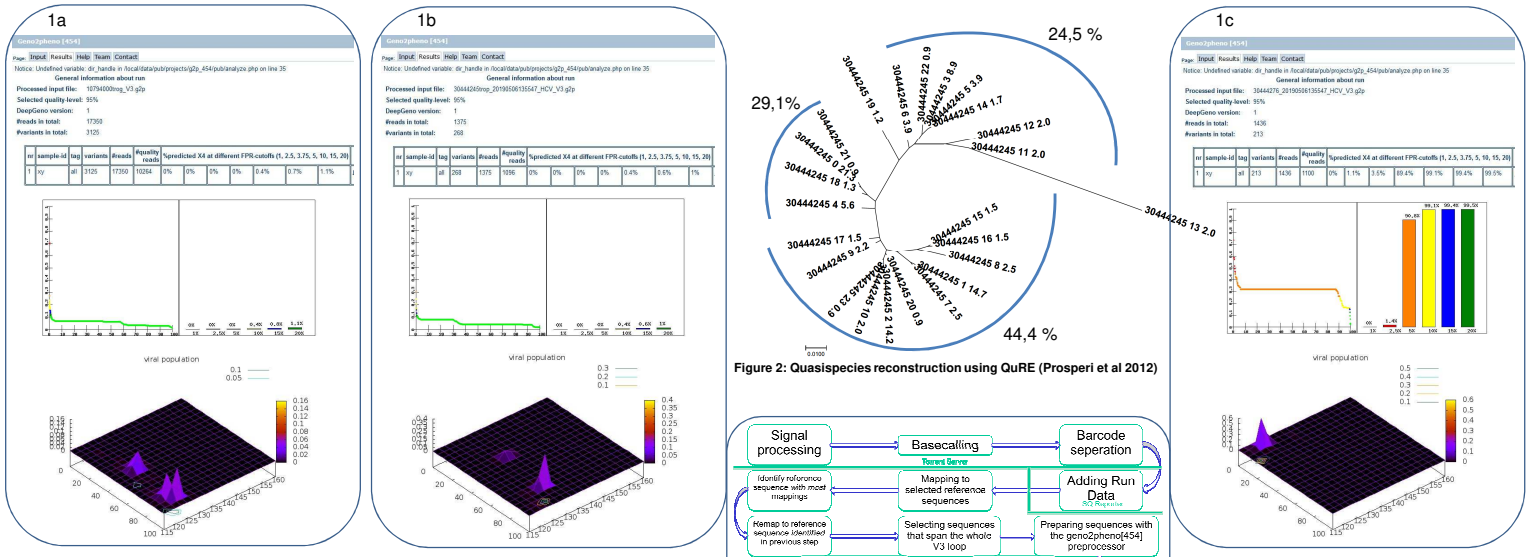

Figure 1 a-c: geno2pheno [454] interprtation for three different samples (a, b and c) including:
the result table on top with predicted %X4 at different FPR-cutoffs
the plot predictions for all reads and colums of predicted %X4 below different FPR-cutoffs
a viral populatioin 3D plot (one axis describes the predicted FPR (0-100); the second a score of difference to something like a V3 consensus sequence(120-160); the z-axis determines the number of reads in this area)

Figure 2: Quasispecies reconstruction using QuRE (Prosperi et al 2012)

Figure 3: MIB NGS tropism pipeline for Vela

RESULTS

Interpretation by geno2pheno [454] led to quality reads from 1000 – 11000 per sample with up to 1700 variants in a single sample. The predicted X4-tropic viruses at the false positive rate (FPR) cut-off of 3.75% varied between 0% and 99% interpreted as X4-tropic only or R5-tropic virus only. Results for proviral and plasma-viral preparations (for samples with viral loads below 200 c./mL or above 200 c./mL) led to comparably result quality. In figures (1a-c) we show examples of geno2pheno[454] analysis of 3 patients with different characteristics. In sample A more than 10000 sequences were interpreted after quality filtering showing multiple CCR5-tropic populations. In sample B a lower amount of sequences were interpreted after quality filtering leading to 3 distinct populations (see also quasispecies reconstruction figure 2). In sample C most of the analysed sequences were typical for a CXCR4 tropic virus.

CONCLUSIONS

Integrating a genotypic tropism test into the Vela Sentosa HIV genotyping and resistance assay showed excellent performance. Integrating the geno2pheno[454] web service into our NGS analysis pipeline proved to be easy and showed reproducible results. The V3-loop sequences could be reliably detected and showed comparable results in geno2pheno 454 analysis compared to next generation sequencing using the illumina platform (data available on request). The combined V3-loop sequencing with the Vela Sentosa HIV genotyping and resistance assay allows short turn-around times and completes the resistance analyses for HIV on the Vela platform.